**Active multiple myeloma suppresses and typically eliminates coexisting MGUS.**

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**ABSTRACT**

**Background:** Myeloma is consistently preceded by premalignant MGUS. In >5% of MGUS patients there is a second MGUS clone (BGUS), yet, at myeloma diagnosis, presentation of biclonal gammopathy myeloma (BGMy) is considered less frequent, implying that myeloma eradicates coexisting MGUS.

**Methods:** In the largest study of its kind, we assessed BGMy frequency amongst 6399 newly diagnosed myeloma patients enrolled in recent UK clinical trials.

**Results:** Compared to expected prevalence (i.e., >5% of MGUS have BGUS), only 58 of 6399 (0.91%) newly diagnosed myeloma patients had BGMy, indicating myeloma typically eliminates coexistent MGUS. In these 58 BGMy cases, the MGUS plasma cell clone was greatly suppressed in size compared to typical levels observed in conventional MGUS; contrarily, the MGUS clone did not inhibit the myeloma plasma cell clone in BGMy.

**Conclusions:** Myeloma eliminates the majority of competing MGUS, and when it does not, the MGUS clone is substantially reduced in size.

## INTRODUCTION

Myeloma and the asymptomatic precursor that consistently precedes it, monoclonal gammopathy of undetermined significance (MGUS) (Landgren *et al*, 2009; Weiss *et al*, 2009), are characterised by monoclonal plasma cells in the bone marrow and monoclonal antibody (MAB) in blood. In a population based study of 12,482 persons, prevalence of MGUS was 3.7% in blacks, 2.3% in whites and 1.8% in Hispanics, and within these MGUS populations, the prevalence of two MGUS clones – termed biclonal gammopathy of undetermined significance (BGUS) (Kyle *et al*, 1981) – was 15.4%, 6.8% and 12.8%, respectively (Landgren *et al*, 2014). A proportion of new myeloma diagnoses also exhibit two MABs in immunofixation electrophoresis (IFE), termed myeloma with biclonal gammopathy (BGMy). This represents progression of one BGUS clone to active myeloma and the continued presence of a secondary MGUS clone (Kyle *et al*, 2003; Kyle *et al*, 1981). Given that the prevalence of MGUS in the general population – at the mean age of myeloma diagnosis – is ~5% (Dispenzieri *et al*, 2010; Wadhera & Rajkumar, 2010), and prevalence of BGUS amongst all MGUS is greater than 5% (Landgren *et al*, 2014), one would also expect more than 5% of myeloma cases to be BGMy. The frequency of BGMy remains uncertain (Garcia-Garcia *et al*, 2015; Guastafierro *et al*, 2012; Kyle *et al*, 1981; Nilsson *et al*, 1986; Riddell *et al*, 1986). In a review of 1,027 myeloma diagnoses in one centre, 2% of cases were BGMy (Kyle *et al*, 2003). This lower than expected figure warrants validation as it implies that when myeloma arises in an individual with BGUS, the malignant myeloma clone eliminates the competing MGUS clone. Here, in the largest study of its kind, we assessed 6,399 newly diagnosed myeloma patients entered into three multi-centre UK clinical trials to determine the frequency, and MAB sizes of, BGMy at myeloma diagnosis.

## MATERIALS AND METHODS

### Trial patients

BGMy patients were identified from 6,399 newly diagnosed myeloma patients enrolled in one of the following multi-centre, phase III trials: the UK MRC Myeloma IX trial (N=1693; ISRCTN68454111); the CRUK Myeloma XI trial up to an induction randomisation date of 23 July 2015 (N=3880; ISRCTN49407852); or, the UK NIHR TEAMM Trial up to a randomisation date of 23 July 2015 (N=826; ISRCTN51731976).

### Laboratory tests

MABs in serum were identified by IFE (Sebia, France) and quantified by protein zone electrophoresis and densitometry (SPE; Interlab, Italy); please refer to Supplementary for more information. In patients with a light chain (LC) MAB without a heavy chain (HC) component (i.e., light chain only myeloma), MAB size was measured and monitored by involved free light chains (iFLCs; Binding Site, UK) expressed in g/L.

### Statistical analyses

Differences between groups were analysed by one-way ANOVA for continuous variables (e.g. MAB size), and Chi-Square for categorical variables and frequencies. One sample t-tests were used to compare observed MAB sizes to previously observed mean MAB sizes in the literature; observed BGMy MAB sizes were compared to reported MAB sizes in MGUS (Turesson *et al*, 2014) and myeloma using a combined dataset from MIX and MXI UK trials. Correlational analyses were conducted using Pearson's for normally distributed data and Spearman's Rank where data was skewed. Significance was accepted at $p<.05$. Data were analysed by IBM SPSS statistics version 21.

## RESULTS

### Frequency of BGMy and demographics of BGMy patients

58 patients had BGMy (from MIX=18; MXI=33, TEAMM=7) and 6341 had monoclonal gammopathy myeloma (MGMy) giving a BGMy frequency of 58/6399 patients (0.91%; 99% confidence interval: ±0.3%). Comparison of the 58 BGMy patients with MGMy patients in the same trials (Heaney et al., in submission) found no differences in age: BGMy median age [range] was 69 [45-86] years, and MGMy was 66 [28-90] years.

### Distinguishing MGUS from myeloma MABs

The frequencies and concentrations of serum MABs in all 58 BGMy patients at trial entry are presented in Table 1; the largest MAB is classified as the myeloma MAB and is referred to as 'M1', whereas the smaller MAB was classified as the MGUS MAB and is referred to as 'M2'. In 4 patients with a FLC MAB >500mg/L and intact immunoglobulin < 5g/L, the FLC MAB was selected as M1. M1s were 10- to 20-fold larger than M2s with median M1 IgG 36.1 g/L compared to M2 IgG 2.6 g/L; and M1 IgA 24.1 g/L compared to M2 IgA 1.7 g/L.

M1 and M2 MAB combinations in BGMy are presented in Table 2 where it can be seen that 37/58 BGMy (65%) combinations exhibited different LC isotypes; assessment of available serum FLC levels in these patients (illustrated in Supplementary Figure 1) demonstrated that elevated FLC levels were predominantly associated with the M1 clone.

In Table 2, we show that two independent IgG MABs was the most common M1 and M2 combination (16/58 pts), but not as common as expected (27/58), and we did not observe any patients with two IgG MABs with the same LC isotype (15/58 expected; $p<0.001$; $\chi 2=17.228$). In 6 BGMy patients with two IgG MABs, the electrophoretic mobilities of the MABs were very similar and so these MABs were only reliably distinguishable by their different LC isotype. Accordingly we

may have missed a third of the 15/58 expected IgG MAB pairs of the same LC isotype, because of electrophoretic mobility similarities.

**MGUS MAB isotypes and levels in BGMy compared to conventional MGUS**

The most frequent M2 was IgG (33/58) and a quarter of M2 clones (14/58) secreted IgM. The frequency of different M2 HC isotypes was closest to the patterns observed in two MGUS studies of 728 and 694 MGUS patients, rather than the different HC frequencies seen in myeloma (Kyle *et al*, 2006; Turesson *et al*, 2014) (Table1). There were, however, less IgG M2s than would be expected in a typical MGUS population (33 versus 41) and more IgM and IgA isotypes (25 versus 16) (trend observed: $p$=0.092 $\chi^2$=2.83).

The level of the MGUS associated MAB (M2) in BGMy was smaller than sizes reported in MGUS (10, 11) by 2.5-fold for IgG and 5-fold for IgA and IgM MABs ($p$<0.001). Thus, whereas coexistent MGUS (i.e., M2) did not appear to suppress the myeloma clone (i.e., M1) in BGMy, the presence of myeloma (M1) significantly suppressed MGUS (M2) MAB levels. The magnitude of this suppression is illustrated in Table 3 where 83% of M2's were below 5 g/L compared to the observed 24% in a prior MGUS study.

**Serum levels of myeloma MABs are the same in MGMy and BGMy**

IgG was the most prevalent M1, followed by IgA, FLC and then IgD; no IgM or IgE M1's were observed; please refer to Supplementary for more information. HC frequencies were very similar to those of 3248 patients in the same clinical trials who had MGMy (Table 1). Notably, IgG and IgA M1 MAB concentrations were not significantly different to those observed in the MGMy patients from the same trials. This indicates that the presence of a second neoplastic plasma cell clone (i.e., M2) does not competitively suppress the expansion of the neoplastic myeloma clone (i.e., M1) (Table 3).

## DISCUSSION

This is the largest study of BGMy frequency in a large cohort of newly diagnosed myeloma patients and demonstrates that myeloma eliminates or greatly suppresses coexisting MGUS. As the prevalence of MGUS in the general population – at the typical age of myeloma diagnosis – is ~5% (Dispenzieri *et al*, 2010; Wadhera & Rajkumar, 2010) and the prevalence of BGUS amongst all individuals with MGUS is >5% (Landgren *et al*, 2014), it would also be expected that >5% of myeloma cases would be BGMy. However, despite rigorous central laboratory analysis, it was found to be just 0.91%.

This shortfall is unlikely to be the result of myeloma being 5-fold more likely to arise in individuals with a single MGUS clone than from individuals with two MGUS clones. A recent study reported the rate of progression from BGUS to myeloma was approximately 1% per year, which is similar to the incidence of MGUS progression to myeloma (Mullikin *et al*, 2016). Thus, when myeloma arises from BGUS, in approximately 80% of cases, the other MGUS clone must be eliminated or suppressed below the limits of detection on IFE (0.1g/l).

In the BGMy cases reported herein, MGUS associated MAB levels were smaller than expected levels typically observed in conventional MGUS (Turesson *et al*, 2014), by 2.5-fold for IgG and 5-fold for IgA and IgM. The degree of suppression of MGUS clones in BGMy appears comparative to the immunoparesis of non-malignant polyclonal plasma cells (PC) that occurs in the majority of newly diagnosed myeloma patients (Kastritis *et al*, 2014; Pruzanski *et al*, 1980; Wang & Young, 2001; Wangel, 1987). Of 3,248 MGMy patients from the same Myeloma IX and XI trials reported in this study, we found that polyclonal immunoglobulin levels were below the normal range in >80% of patients and levels were lower than the median level found in healthy controls by 2.5-fold for IgG, and 7-fold for IgA and IgM (Heaney *et al*, Submitted). Despite their larger relative presence, MGUS clones appear to only compete effectively with polyclonal PC in ~20% of cases, as evidenced by incidence of immunoparesis in MGUS (Turesson *et al*, 2014). As only a fifth of

MGUS clones exhibit competitiveness for the normal PC niche, we hypothesise that these are the only MGUS clones that survive when myeloma arises in the wider marrow environment and that they are subject to the same suppression of clonal size and antibody secretion as normal polyclonal plasma cells; this would also be applicable to the MGUS PC clone from which myeloma arose. In a separate observation, we observed in this study that two IgG MABs was the most common BGMy combination (16/58 pts) but not as common as expected (27/58) indicating that the presence of IgG myeloma PC clones suppresses IgG MGUS PC clones more than IgA or IgM PC clones. There is evidence from both MGUS and myeloma that neoplastic IgG PC clones exert a greater degree of suppression on normal polyclonal IgG PC than they do on IgA and IgM isotypes (Bradwell *et al*, 2013; Katzmann *et al*, 2013; Ludwig *et al*, 2016).

A limitation of our study is that we were unable to conduct longitudinal measurement of BGUS prior to myeloma diagnosis; this excluded the possibility of investigating elimination of MGUS clones at the time of myeloma clone proliferation. Future prospective studies may seek to explore differences between BGUS which progress to myeloma, and BGUS that do not progress to myeloma, and measurements should incorporate bone marrow tumour samples as well as stroma to investigate possible mechanisms of tumour eradication. In summary, our findings confirm that BGMy is rare, and the survival of a coexisting MGUS clone is hallmarked by reduced MAB size.

## Conflict of Interest Statement

All authors declare no conflict of interest.

## Author Contributions

JC, CB and MD wrote the manuscript. MD, JC and JH designed the investigation into BGMy. JC, JH, SP, and ZA conducted the experiments. JC, JH, SP AND MD interpreted the data. MK, RO, GM, GH, AC and MD designed the Myeloma IX, XI and/or TEAMM trials. All authors approved the manuscript

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**Table 1.** Characteristics and frequencies of M1 and M2 in 58 BGMy patients at myeloma diagnosis, compared to expected frequencies and characteristics of MGMy and conventional MGUS.

| Monoclonal Antibody (MAB) 1 | | | | | | | Monoclonal Antibody (MAB) 2 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HC Isotype | | Frequency (*N*) | Proportion (%) | Conc. (g/L) (median [range]) | MGMy reference ranges † Proportion (%) | MGMy reference ranges † Conc. (g/L) (median [range]) | HC Isotype | | Frequency (*N*) | Proportion (%) | Conc. (g/L) (median [range]) | MGUS reference ranges ‡ Kyle et al., 2006 Proportion (%) | MGUS reference ranges ‡ Turesson et al., 2013 Proportion (%) | MGUS reference ranges ‡ Turesson et al., 2013 Conc. (g/L) (median ± SD) |
| IgG | Total | 39 | 67.2 | *36.1 [4.8-70.0] | 59.9 | 34.4 [0.8-100.2] | IgG | Total | 33 | 56.9 | **2.6 [0.5-12.0] | 68.9 | 68.8 | 7.0 ± 6.0 |
| | IgGκ | 26 | 66.7 | 32.6 [4.8-70.0] | | | | IgGκ | 17 | 51.5 | 2.5 [0.5-12.0] | | | |
| | IgGλ | 13 | 33.3 | 39.7 [10.2-60.0] | | | | IgGλ | 16 | 48.5 | 2.8 [0.5-9.0] | | | |
| IgA | Total | 14 | 24.1 | *25.4 [1.8-60.0] | 23.6 | 33.0 [0.2-96.6] | IgA | Total | 11 | 19.0 | **1.7 [0.5-11.6] | 10.8 | 14.7 | 8.0 ± 5.0 |
| | IgAκ | 8 | 57.1 | 26.9 [1.8-60.0] | | | | IgAκ | 5 | 45.5 | 1.3 [0.5-3.9] | | | |
| | IgAλ | 6 | 42.9 | 23.2 [3.9-53.0] | | | | IgAλ | 6 | 54.5 | 2.5 [0.5-11.6] | | | |
| IgD | Total | 1 | 1.7 | 2.6 [2.6-2.6] | 1.7 | 4.2 [0.2-36.9] | IgM | Total | 14 | 24.1 | **1.3 [0.5-6.0] | 17.2 | 16.2 | 7.0 ± 6.0 |
| | IgDκ | 0 | 0.0 | - | | | | IgMκ | 12 | 85.7 | 1.3 [0.5-6.0] | | | |
| | IgDλ | 1 | 100 | 2.6 [2.6-2.6] | | | | IgMλ | 2 | 14.3 | 1.6 [0.6-2.7] | | | |
| FLC | Total | 4 | 6.9 | 1.0 [0.5-1.9] | 13.1 | 2.6 [0.02-46.7] | | | | | | | | |
| | FLCκ | 3 | 75.0 | 1.4 [0.5-1.9] | | | | | | | | | | |
| | FLCλ | 1 | 25.0 | 0.7 [0.7-0.7] | | | | | | | | | | |
| LC Isotype | | Frequency *(N)* | Proportion (%) | Conc. (g/L) (median [range]) | MM reference proportion † (%) | | LC Isotype | | Frequency *(N)* | Proportion (%) | Conc. (g/L) (median [range]) | MGUS reference proportion (%) Kyle et al., 2006 | | |
| κ | | 37 | 63.8 | 26.0 [0.5-70.0] | 66.0 | | κ | | 34 | 58.6 | 1.8 [0.4-12.0] | 62.0 | | |
| λ | | 21 | 36.2 | 27.0 [0.7-60.0] | 34.0 | | λ | | 24 | 41.4 | 2.7 [0.5-11.6] | 37.9 | | |

† Reference ranges aggregated from 3248 patients with MGMy diagnosed at entry into Myeloma IX and Myeloma XI trials; dataset cut-off June 2013.
‡ Reference ranges derived from 728 MGUS patients (Turesson et al, 2013), or 694 MGUS patients (Kyle et al, 2006).
* Significant difference between M1 and M2 concentration (IgG $p<0.001$; IgA $p<0.001$)
** Significant difference between M2 concentration and expected MAB concentration in MGUS (IgG $p<0.001$; IgA $p<0.001$; IgM $p<0.001$) (Turesson et al, 2013). No significant differences observed between M1 and expected MAB concentrations in MGMy ($p>0.05$) based on observations from Myeloma IX and XI.

**Table 2.** Frequency of M1 and M2 combinations observed in BGMy.

| MAB 1 | MAB 2 | MAB 2 Frequency | | MAB 2 Proportion | |
|---|---|---|---|---|---|
| | | Observed BGMy frequency *(N)* | Expected MGUS† frequency *(N)* | Observed BGMy proportion (%) | Expected MGUS† proportion (%) |
| IgG κ [*N*=26] | IgG κ | 0* | 12 | 0 | 46 |
| | IgG λ | 10 | 6 | 38 | 23 |
| | IgA κ | 3 | 3 | 12 | 10 |
| | IgA λ | 4 | 1 | 15 | 5 |
| | IgM κ | 7 | 3 | 27 | 11 |
| | IgM λ | 2 | 1 | 8 | 5 |
| IgG λ [*N*=13] | IgG κ | 6 | 6 | 46 | 46 |
| | IgG λ | 0 | 3 | 0 | 23 |
| | IgA κ | 2 | 1 | 15 | 10 |
| | IgA λ | 2 | 1 | 15 | 5 |
| | IgM κ | 3 | 1 | 23 | 11 |
| | IgM λ | 0 | 1 | 0 | 5 |
| IgA κ [*N*=8] | IgG κ | 5 | 4 | 63 | 46 |
| | IgG λ | 1 | 2 | 13 | 23 |
| | IgA κ | 0 | 1 | 0 | 10 |
| | IgA λ | 0 | 0 | 0 | 5 |
| | IgM κ | 2 | 1 | 25 | 11 |
| | IgM λ | 0 | 0 | 0 | 5 |
| IgA λ [*N*=6] | IgG κ | 4 | 3 | 67 | 46 |
| | IgG λ | 2 | 1 | 33 | 23 |
| | IgA κ | 0 | 1 | 0 | 10 |
| | IgA λ | 0 | 0 | 0 | 5 |
| | IgM κ | 0 | 1 | 0 | 11 |
| | IgM λ | 0 | 0 | 0 | 5 |
| IgD λ | IgG κ | 1 | | | |
| LCO κ | IgG λ | 3 | | | |
| LCO λ | IgG κ | 1 | | | |

† Reference range proportions obtained from Turesson et al, (2014), with assumed 2:1 proportion of kappa:lambda for each heavy chain isotype.

Note: Due to relatively low frequency, data from 1 patient with IgD MAB 1, and 3 patients with FLC MAB 1 not shown.

* Chi-Squared analyses revealed significant differences between the observed frequency of IgGκ pairs in BGMy compared to those expected in MGUS ($p$<0.001; $\chi^2$=15.60). A trend ($p$=0.065; $\chi^2$=3.391) was observed for frequency of observed IgGλ pairs in BGMy and those expected in MGUS.

**Table 3.** Comparison of M1 and M2 concentrations in BGMy with expected MAB sizes in conventional MGMy, and MGUS.

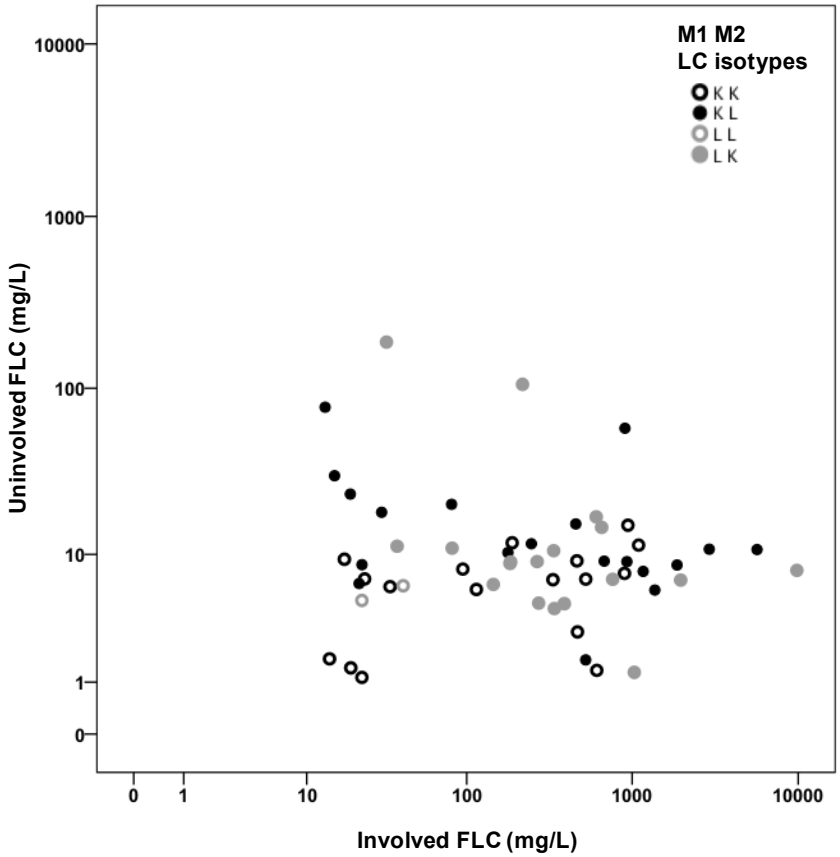
| Size | M1 Proportion & Frequency* | Expected proportion in MGMy †** | M2 Proportion & Frequency | Expected proportion in MGUS ‡ |
|---|---|---|---|---|
| <4.99g/L | 7% [*N*=4] | 7% | 83% [*N*=48] | 24% |
| 5.00g/L - 9.99g/L | 7% [*N*=4] | 5% | 14% [*N*=8] | 19% |
| 10.00g/L - 14.99g/L | 7% [*N*=4] | 5% | 3% [*N*=2] | 33% |
| 15.00g/L - 19.99g/L | 7% [*N*=4] | 6% | 0% [*N*=0] | 18% |
| 20.00g/L - 24.99g/L | 13% [*N*=7] | 9% | 0% [*N*=0] | 5% |
| >25.00g/L | 57% [*N*=31] | 68% | 0% [*N*=0] | 1% |

† Reference range represents aggregated data from patients with MGMy diagnosed at entry into Myeloma IX and Myeloma XI trials.

‡ Reference range derived from 694 MGUS patients (Kyle et al, 2006).

*Excludes 4 patients diagnosed with a FLC myeloma M1 [all 4 patients had a whole MAB < 4.99 g/L)

**Excludes patients with FLC myeloma and non-secretory myeloma.




**Supplementary Figure 1**. FLC levels at disease presentation in BGMy patients with FLC results available (N=56 of 58 patients). Patients are colour coded according to the LC-isotypes of M1 and M2, respectively. The involved FLC was identified by the LC isotype of M1.

## SUPPLEMENTARY METHODS

### Laboratory testing

If accurate quantitation of MABs was not feasible – e.g., when a pair of MAB bands shared the same position on SPE (i.e., IgGκ IgGλ), or when the size of the MABs were too small to be detected by densitometry (limit of detection: ~1g/L) – MAB concentration was estimated from IFE, taking into account the size of the monoclonal bands as a proportion of total immunoglobulin of that HC isotype (i.e., taking into account background polyclonal immunoglobulin); this exercise was carried out by three experienced IFE users, independently, blind of sample timepoint, before agreement was reached per sample.

## SUPPLEMENTARY RESULTS

### MABs that may have been missed by IFE

In this study, HC isotype frequencies for the M1 MABs were very similar to those in patients in the same clinical trials who had MGMy. The exception was a reduced number (4 versus 8) of expected FLC myeloma due to the exclusion of patients with a FLC and an intact immunoglobulin MAB exhibiting the same LC isotype because the MABs were presumed to derive from the same PC clone. Further, as discussed elsewhere, we may have missed a third of the 15 expected IgG MAB pairs exhibiting the same LC isotype because of very similar electrophoretic mobility. Similarly we may have missed 2 IgA BGMy patients. These three problems in determining the presence of MABs from two separate PC clones are likely to have reduced the numbers of BGMy detected. However the same problems apply equally to identification of BGUS and so the discrepancy between prevalence of BGUS in MGUS patients and prevalence of BGMy in myeloma patients remains at five fold.